

## PERSPECTIVE

For the last several years, improvements in endotoxin testing have come predominantly in the areas of incubating readers, kinetic software, and lab automation. Although these improvements were helpful in meeting compliance requirements, managing data, and increasing a lab's throughput, they offered very little to truly reduce the complexity of the assay and to generate faster results for critical samples. We have seen over the years a continued trend to centralize testing in-house or to use contract test facilities. Centralized testing has some great advantages when it comes to the investment in capital equipment, the calibration/validation processes, data management and cost control. There are some critical disadvantages, however, specifically with regard to the time for sample turnaround and the training and availability of technicians trained to do LAL (Limulus Amebocyte Lysate) assays. Charles River understands the need for rapid turnaround on testing and for ease-of-use when performing the assays. We have developed kinetic LAL reagents that are the fastest and most interference-resistant in the industry. Our software is user friendly, secure, and offers excellent flexibility. Several years ago, however, we determined that continued improvements to the existing kinetic LAL testing format would do nothing to improve ease-of-use and sample turnaround time. We then set out to do something extraordinary. This newsletter announces Charles River's revolutionary new portable endotoxin detection system that was developed with a dramatic change in LAL test platforms. We are very proud to introduce **ENDOSAFE®-PTS**.

## ENDOSAFE®-PTS (Portable Test System)

Our vision is now reality: From the outset we envisioned an LAL assay that was easy to use, portable and fast. We realized that by placing the actual testing in the hands of the technicians collecting the samples, we could achieve real time analysis with direct test results available at the point of sample collection. Our mission is to decentralize the testing while simultaneously maintaining centralized QA controlled data management. This can be achieved by performing the assays immediately at the point of collection and then delivering the test data to a central database (such as LIMS) for evaluation and archival.

We have completed development of the necessary instrumentation and cartridges for performing a point of collection assay. Since the Endosafe®-PTS is currently under evaluation by the FDA, it can only be used for samples not requiring a licensed LAL product. This does not however diminish the value the existing PTS platform has as an early endotoxin assessment tool for critical production samples. PTS can also be used to provide researchers with quick results because the testing never leaves their hands. Once FDA has approved the new technology, these early indicator tests will become the official real time endotoxin test results for all in process samples. Ultimately, the handheld PTS units will send all testing data to a centralized database for evaluation and recording.

## ENDOSAFE®-PTS TECHNOLOGY

The Endosafe®-PTS system utilizes several patent-protected and patent-pending technologies with regard to LAL methodology, instrumentation, and reagent preparation.

### Novel Kinetic LAL Assay

Existing LAL technology consists of three basic methods: gel-clot, endpoint, and kinetic assays. The qualitative gel-clot reagent is the oldest technology and is performed by incubating the LAL reagent with standards/samples for 60 minutes in glass test tubes and then reading the results by inverting the tubes 180°. Samples are positive for endotoxin if a gel is present in the tube and negative if no gel is present.

The endpoint chromogenic method consists of the following steps:

- 1) Mix the LAL reagent with standards/samples
- 2) Incubate the mixture at 37°C for a specified time
- 3) Add a synthetic chromogenic substrate that turns yellow in the presence of endotoxin
- 4) Additional incubation at 37°C
- 5) Stop the reaction with acid

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A linear relationship exists between endotoxin concentration and absorbance values. Therefore regression analysis and subsequently unknown endotoxin quantification can be performed utilizing Beer's Law. Although the endpoint assay works well and is very quick, its range is limited to one log.

The kinetic methods utilize more sophisticated kinetic spectrophotometers to monitor either turbidity (turbidimetric) or color formation (chromogenic). A linear relationship exists between the log of endotoxin concentration and the log of reaction time. Reaction time is the time in seconds required for the standard/sample to change by a specified optical density (OD). The current kinetic assays offer a wide range of up to 4 logs.

The PTS is a two stage kinetic assay that offers similar ranges and sensitivity as the most sophisticated kinetic LAL assay, but it has one very significant advantage - TIME. The new two stage kinetic test detects endotoxin over the same endotoxin ranges, but in much less time, as shown below.

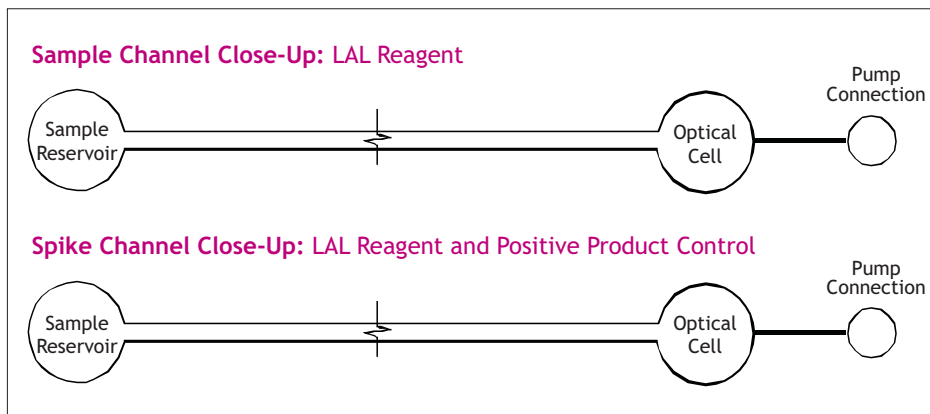
Method	Range	Sensitivity	Assay Time	Linearity
Kinetic	5-0.05 EU/ml	0.05 EU/ml	32 minutes	-0.999
Endpoint	1-0.1 EU/ml	0.1 EU/ml	15 minutes	0.999
PTS	5-0.05 EU/ml	0.05 EU/ml	19 minutes	-1.00

### Reader Portability

The PTS reader consists of an incubating chamber, sample pump, four LEDs and detectors, alpha numeric key pad with built-in LCD, and microprocessor. The reader operates using standard AC power or on an internal rechargeable battery so a user can easily move from one sample collection point to the next, getting endotoxin readings along the way.

### Disposable LAL Reagent Cartridges

The cartridge used in the PTS test is a single use, polystyrene cartridge



loaded with licensed LAL reagent and endotoxin controls. The cartridge contains two sample channels and two spiked channels consistent with current FDA licensed quantitative LAL methods. The PTS chamber maintains the required temperature and the optical density is read upon completion of the test.

### Performing a PTS Assay

Using the Endosafe®-PTS is simple. The reader prompts the user through the necessary steps:

- 1) Remove cartridge from pouch and insert into reader
- 2) Key pad entries:
  - a) User Operator ID: User Name
  - b) Cartridge Lot #: wxyz
  - c) Calibration Code: \*123456789101112
  - d) Verify Archived Curve Range: "Enter"
  - e) Sample Lot #: abcd
  - f) Sample Name ID: WFI
  - g) Dilution Factor: 1
  - h) Add sample and push the "Enter" button on the PTS keypad

*\*If a calibration code has previously been entered for the lot of cartridges being used, it will skip this prompt.*

### Obtaining LAL Test Results

After the assay is complete, the results will appear on the LCD for instant evaluation:

Sample value: <0.05 EU/ml  
 CV: 13.2 %  
 Spike recovery: 120%  
 CV: 7.8%

These results will be stored in memory in the reader (for up to 100 individual assays) and can be printed or exported to another program at a later time.

### LAL POINTERS

One of the most common problems associated with kinetic LAL testing is the lack of proper controls used in the selection of accessory products used to conduct the assay. The accessories include but are not limited to LRW, endotoxin dilution tubes, pipettes, pipette tips, microplates and sample containers. Since PTS cartridges are already preloaded with all the required reagents, it is only necessary that the sample be added to the cartridge aseptically. This will require pyrogen-free tips and sample containers. This test configuration dramatically reduces the opportunity for inappropriate accessory products to interfere with the assay. Should it be necessary to perform dilutions on the samples, Charles River offers a full line of pyrogen-free accessories.

## LABORATORY NOTEBOOK

### Comparison of methods

The Endosafe®-PTS is comparable to current kinetic LAL methods.  
The table below demonstrates equivalency to existing kinetic methods.

Sample	Method	Dilution	Endotoxin Value	Spike Recovery	Test Time (min.)
WFI	KTA	1	< 0.05 EU/mL	82%	41
	KCA	1	< 0.05 EU/mL	80%	36
	PTS	1	< 0.05 EU/mL	68%	16
3% NaCl	KTA	1:20	< 1.0 EU/mL	85%	41
	KCA	1:20	< 1.0 EU/mL	77%	36
	PTS	1:20	< 1.0 EU/mL	111%	16
20% HSA	KTA	1:10	< 0.5 EU/mL	undefined	41
	KCA	1:10	< 0.5 EU/mL	10%	36
	PTS	1:10	< 0.5 EU/mL	55%	16
Protein Sample*	KCA	1:1000	845.9 EU/mL	127%	37
	PTS	1:1000	614.0 EU/mL	103%	16

\*Did not run with KTA.

In another comparison, various contract testing samples were run by the requested test method - kinetic chromogenic, kinetic turbidimetric and gel-clot - as well as by the PTS method. The data from this analysis using a range of random sample types, show equivocal results for the PTS with all of the LAL test methods employed.

Sample	Method	Dilution/ Concentration	Endotoxin Value	Spike Recovery
Sample A	KCA	1:100	26.0 EU/mL	116%
	PTS	1:100	33.7 EU/mL	95%
Sample B	KCA	1:1000	388.2 EU/mL	94%
	PTS	1:1000	279.0 EU/mL	92%
Sample C	KTA	1:20	2.7 EU/mL	100%
	PTS	1:20	1.7 EU/mL	111%
Sample D	Gel-clot	1:100,000	3,125 EU/mL	Valid
	PTS	1:100,000	3,950 EU/mL	149%
Sample E	Gel-clot	1:5	<0.15625 EU/mL	Valid
	PTS	1:5	<0.25 EU/mL	59%

*The Endosafe®-PTS (Portable Test System) is a rapid, point-of-use test system that utilizes FDA-licensed LAL formulations loaded into a test cartridge along with a handheld spectrophotometer. The PTS provides quantitative LAL test results in about 15 minutes with a simple, one button operation. Results are displayed on the screen and can be printed or downloaded for reporting and trending.*

## WHAT'S NEW

### Charles River Laboratories Annual Summer LAL Workshop:

- **August 19-22, 2003**  
*Kinetic & Gel Clot LAL Test Methods*  
held in Charleston, SC

### New Product Information:

- **Endosafe® - PTS reader**  
PTS 100
- **Endosafe® - PTS cartridge**  
PTS 201/PTS 2005
- **Minipipettor**  
PTS 400
- **Printer**  
PTS 300

To view a video of the PTS system,  
please go to [www.criver.com/pts](http://www.criver.com/pts).

## ENDOSAFE TIMES

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